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The Effect of Aging upon the Cell Population of the Mouse Periodontium

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THE EFFECT OF AGING UPON THE CELL POPULATION OF THE MOUSE PERIODONTIUM

BY

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A Thesis Submitted to the Faculty of the Graduate School of Loyola University in Partial Fulfillment of the Requirements for the Degree of Master of Science

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DEDICATED TO MY MOTHER AND FATHER

LILLIAN AND ADOLPH

LIFE

Melvin B. Borg was born in New York City, October 3, 1939. He completed his secondary school education at Christopher Columbus High School, January, 1957.

He attended New York University for one year and then transferred to the University of Illinois at Urbana, in February, 1958 where he completed his undergraduate studies in June, 1960.

In September, 1960 he began his dental training at the University of Illinois, School of Dentistry. He received a Bachelor of Science Degree in June, 1962 and the degree of Doctor of Dental Surgery in June, 1964 from the University of Illinois.

From July, 1964 to June, 1965 he served on a rotating dental internship at Montefiore Hospital, New York City.

The author began graduate studies in the Department of Oral Biology of Loyola University, Chicago, in June, 1965.

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CHAPTER I

INTRODUCTION

Periodontium studies by Jensen (1966) of the frequency distribution of labeled cells in aging rat periodontium, demonstrated a reduction of labeled cells with age. Pinzon (1965) found a similar reduction of labeled cells with age in the pulp. This study was undertaken to compare the effect age will have upon the number of cells in the periodontal ligament, the frequency of labeled cells preparing to divide and the time required for labeled cells to divide in the mouse.

CHAPTER II

REVIEW OF THE LITERATURE

A. Aging and Connective Tissue:

Everitt (1959) studying the aging process in rats found the metabolic activity of the organism declining with age throughout the life cycle. Heat production, food consumption and heart rate were reduced.

The histologic appearance of cells change with age. Boucek, Noble and Woessner (1959) found that the rat fibroblast became narrower, exhibited a nuclear condensation and decreased amount of cytoplasm in older animals.

Ring (1960) studying rats, Klingsberg and Butcher (1960) studying rats and hamsters, observed in connective tissue a marked reduction in all cell types and increase in fibrous elements. Wentz, Mair and Orban (1952) found a reduction in the cellular elements of human gingival connective tissue and increased fibrosis with increasing age. Belting et al. (1953) studying apposition of alveolar bone in rats, saw a reduction with age and resorption in the senile. Massler (1956) observed the periodontal structures undergoing atrophy and fibrotic

changes in the aged human.

Tonna and co-workers (1958, 1959a, 1959b, 1960, 1961, 1962 and 1966) working with rats and mice, compared the changes in periosteal tissues between young and old animals. In the older animal, the osteoblast became spindle-shaped, having pyknotic nuclei and less cytoplasm. It was sometimes impossible to distinguish them from the fibroblast. Also, in the old animal, there was a progressive depletion in the number of osteoblasts until the old animal had one-tenth the number of the young. Autoradiographic studies using tritiated thymidine further showed that as age increased the number of labeled osteoblasts decreased. The overall proliferative response of the osteogenic cells to the stimulus of a fracture decreased with age. The changes occurring in the osteoblast were not just morphological and numerical, but were also physiological. One such example was the depression of respiratory enzyme activity which makes energy available to the cell. It is important to observe that this occurred prior to observed cytological age changes. Trauma stimulated a response of respiratory activity. However, the response became progressively less in the old animal. At the same time, there was a nine fold decrease in the number of

mitochondria per osteoblast.

Pinzon (1965) in an autoradiographic study of the rat pulp at various ages found a decrease in the number of labeled cells in the older animal.

Klingsberg and Butcher (1960) found the periodontal ligament in rats and hamsters very cellular in the young, while in the aged a decreased cellularity was noted with an increase in the density of the connective tissue fibers.

Jensen (1966) autoradiographically studying cellular proliferation in the periodontal ligament of rats found with an increase in age a decrease in the labeling index.

Lord (1964) found in the young rat the number of labeled cells doubled within twenty-four hours in the periodontal ligament indicating mitosis had occurred.

Decrease in mitoses has been noted with age in other tissues besides connective tissues. Andrew (1949) studying the rat parotid gland found numerous mitotic figures in the young on hematoxylin and eosin stained slides. There was a slowing down of mitotic activity with age. In senile animals there was fatty degeneration of the parenchyma with

no mitotic figures. Walker (1958) examined the rat lacrimal gland on hematoxylin stained slides. Mitotic activity was present at all ages. There was much mitotic activity in the young which became rare in old age. McKellar (1949) investigated mitotic activity in the rat liver. Prior to sacrifice, the animals were injected with colchicine to arrest mitosis. Maximal activity occurred after hemopoiesis ceased. Mitosis then fell rapidly until a low level was reached in the aged. McCreight and Sulkin (1959) studied cellular proliferation in the rat kidney. The slides were stained with fast green in order to study the nuclear detail. The number of mitoses in the senile animal was significantly less than for the young specimens.

B. Autoradiography:

In autoradiography, a photographic emulsion is placed in contact with a slide containing a radioactive substance. The radioactivity forms a latent image on the film that is made evident by photographic developer. Silver grains are then seen lying above the specimen.

Prior to mitosis, there is a duplication of the deoxyribonucleic acid (DNA) present in the nucleus. The cell takes up various DNA precursors, one of these is thymidine and it is available in a tritiated

form. Reichard and Estborn (1951) demonstrated that tritium labeled thymidine (thymidine - H^3) is a specific precursor for DNA and not incorporated into ribonucleic acid (RNA). The use of thymidine - H^3 is therefore a valuable tool for the study of cell turnover in tissues. Evidence that thymidine - H^3 as part of DNA of a chromosome remains intact during succeeding duplications and nuclear divisions was nicely described by Taylor, Woods and Hughes (1957). They showed that DNA turnover is solely the result of mitosis or death. Amano, Messier and Leblond (1959) proved that radioactivity resulting after thymidine - H^3 injection is located solely in DNA.

Messier and Leblond (1960) point out shortcomings are involved in mitotic counts for the study of cell proliferation. In some tissues mitoses are rare, and in others they are barely discernible. After dividing, cells are indistinguishable from the adjacent cells and it is impossible to determine if they remain or move away. These deficiencies can be overcome by radioautography. Labeled cells will continue to emit their radioactivity whether they remain at their site of division or migrate to a new area.

Thymidine - H^3 uptake is very rapid and is available to the tissues

for a brief period. Messier and Leblond (1960) demonstrated the peak value is reached in the plasma about twenty minutes after subcutaneous injection. The level then decreases rapidly. Any radioactivity present in tissues days or months after one injection results from the uptake within the first hour after injection. Rubini et al. (1960) showed that with intravenous administration of thymidine - H^3 the plasma is cleared in the very first circulation of the blood throughout the body, with incorporation into newly formed DNA as early as one minute after injection. About one-third of the thymidine - H^3 was catabolized to tritiated water and nonvolatile tritiated compounds.

Taylor, Woods and Hughes (1957), Beagrie and Skougaard (1962) demonstrated that a labeled cell upon division equally distributes its radioactivity between the two daughter cells.

Tritium emits beta waves. These are low energy and can penetrate only six microns in water or tissue and only two microns in a photographic emulsion. Essentially, all of the silver grains lie clustered within a micron of a labeled nucleus. This results in an autoradiograph of extremely fine resolution, Hughes (1958) and Tonna (1961).

CHAPTER III

METHODS AND MATERIALS

Forty male Swiss albino mice were used in this experiment. They were divided into two groups of twenty animals each. One group was 60-days-old, the other 300-days-of-age. Both groups are considered adult animals, one young adult, the other aged. At these ages, all the molar teeth have erupted and are in occlusion with their antagonists. Until the time of sacrifice, the animals were given food and water ad libitum.

Each animal was injected intraperitoneally with tritiated thymidine at a dose level of 1.0 microcurie per gram of body weight. The specific activity was 1.9 curies per millimole.

At two hour intervals, after injection, one animal was sacrificed, by an overdose of ether, in each group, through forty hours. Both groups of animals were divided into two divisions. The animals sacrificed before twenty hours following injection were compared to the animals sacrificed after twenty hours following injection.

The maxilla was removed and immediately placed into ten percent neutral buffered formalin for fixation. The specimens were decalcified

with formic acid - sodium citrate solution, dehydrated with ascending ethyl alcohol, cleared in xylene, and embedded in paraffin. All of the central sections were cut through the first molar at a thickness of five microns. Two sets of sections from each animal were stained with hematoxylin and eosin.* Another six sets of the sections from each animal were prepared as autoradiograms. The latter slides were coated with Kodak nuclear track emulsion type NTB3 and subsequently stained with Nuclear Fast Red and Indigo-Carmin.**

Central sections were cut through the maxillary first molar tooth. Selected for detailed histologic study was the mesial surface of the mesial root. The exact area involved was taken from a horizontal line at the alveolar crest to the cementum of the tooth, to a vertical line from the apex of the tooth to the alveolar bone. The best central sections obtained were studied. Any specimen not having this entire periodontal area intact was discarded.

A Whipple disk was placed in the ocular of the microscope, to

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*Ann Preece, A Manual for Histologic Technicians, 2nd ed. (Boston, 1965), pp. 153-158.

**Micheline Mortreull-Lauglois, Department of Comparative Anatomy and Histology, Faculty of Science, University of Paris, (Paris).

divide the field of view into compartments, and thus facilitate cell counting. Two representative sections stained with H&E were selected from each animal. All the connective tissue cells of the periodontium were then counted with the exception of endothelial cells and blood cells. The total was divided by two to obtain an average cell count for the individual animal. In this manner, variations due to the angle of sectioning the individual specimen of each animal would be eliminated.

On the autoradiographic slides, the labeled cells of the periodontium were counted with the exception of endothelial cells and blood cells. Six representative sections were selected at random from each animal. The total labeled cell count was divided by six to obtain an average. The average number of labeled cells was divided by the average number of cells present in the periodontium and multiplied by one hundred to obtain a percentage index of labeled cells.

Before a cell was considered as being labeled, it had to meet the following criteria:

1. The grains had to be clustered over a nucleus.

2. There had to be three grains or more above background radiation.

CHAPTER IV

FINDINGS

A general observation made upon histologic examination of the slides was that the majority of labeled cells excluding endothelial cells and blood cells in the area of the periodontium studied, were located near the apex. The area of second greatest concentration was near the alveolar crest although it was hardly as large as the apical region. Furthermore, very few labeled cells were found between the two areas of alveolar crest and apex.

Comparisons were made between the young group and aged group of animals as to the relative number of labeled and unlabeled cells. The average total number of cells located in the mesial surface of the mesial root of the maxillary first molar is 1003.9 for the young group and 689.8 for the aged group (Table I, Figure 1 and Figure 2). The "t test" was applied to determine the statistical significance of this difference and was found to be significant at a confidence level greater than 0.001.

The average of labeled cells per section is 3.95 for the young and 1.35 for the aged group (Figure 3 and Figure 4). This difference is significant to a confidence level greater than 0.001.

The percentage of labeled cells is 0.394 in the young and 0.197 in the aged group. This difference is significant to a confidence level greater than 0.005.

The animals in each age group were equally divided. The first half consisted of animals two to twenty hours after injection. The second half was composed of animals twenty-two to forty hours after injection. In each instance the percentage of labeled cells was notedly less in the first half as compared to the second half. These values were 0.258 and 0.179 for the first half of the young and aged respectively and 0.636 and 0.241 for the second half. The confidence level of the young group was 0.005 and in the aged 0.5 (Table II and Figure 5).

CHAPTER V

DISCUSSION

The number of connective tissue cells located in the periodontal ligament of the mesial surface of the mesial root in the mouse maxillary first molar is significantly greater in the young as compared to the old mouse. This observation agrees with the results of Klingsberg and Butcher (1960) and Jensen (1966).

The cells of the periodontal ligament duplicate their deoxyribonucleic acid (DNA) content prior to mitosis. This supports the observation of Messier and Leblond (1960) in the periodontal ligament of the rat incisor. One of the essential precursors of DNA synthesis is thymidine. When the cells that are actively synthesizing DNA are exposed to tritiated thymidine, they incorporate it, and become radioactive themselves. Thus, by injecting radioactive thymidine into a mouse it is possible to study the frequency of labeled cells in the periodontium. The ratio of the observed number of radioactive labeled cells to the number of nonlabeled cells in the periodontium is expressed as the labeling index and may be presented as percent of labeling.

The cells of the mouse periodontium which synthesize DNA,

containing tritiated thymidine, undergo mitotic division and distribute their radioactivity equally between their two labeled daughter cells. This equal distribution of radioactivity was shown by Beagrie and Skougaard (1962). Consequently, after mitosis a rise in the labeling index occurs in the mouse periodontium.

The regenerable cells in the periodontium of the 60-day-old mouse is significantly greater than that of the 300-day-old mouse. This is made evident by the greater number of labeled cells in the periodontium of 60-day-old mice. It is probable that this greater number of regenerable cells also accounts for the greater cell populations in the periodontium of younger mice.

The ratio of labeled periodontal ligament cells to nonlabeled cells while significantly greater in the young mice, than in the old mice, probably accounts both for growth requirements as well as renewal of the cell population. This is suggested by the observation in the old mice in which the ratio of labeled to nonlabeled cells is only about one-half that in the young mice. All of this assumes that there should exist some equivalence in the ratio of labeled cells in the periodontal ligament of 60-day-old and 300-day-old mice, and that the growth

requirements of the old mice is very small and "needs" only regenerable cells for purposes of renewal.

As the ratio of labeled cells to nonlabeled cells significantly falls in the old mice, the cells of the periodontal ligament do not renew as rapidly as they do in the young mice. Consequently, the cells live longer. The collagen fibers become increasingly more insoluble with age Gross (1961). Also, the ratio of acid mucopolysaccharides to collagen reduces with age Ring (1960). This may cause increased density of the connective tissue resulting in a reduction in the movement and availability of the molecules of DNA precursors in the connective tissue. This could result in a reduction of cells synthesizing DNA and therefore the number of cells able to undergo mitosis.

Neuberger et al. (1951) demonstrated that collagen in old rats is metabolically almost inert with a negligible turnover rate. Kao et al. (1962) showed a lowered capacity of aged tissues for forming collagen. Kao and McGavack (1959) observed the percentage of soluble collagen in total collagen decreased with age. These facts can be explained by the reduced number of cells capable of forming collagen in the old animal. Since there are less cells present there is less new soluble collagen produced.

The labeled cells of the periodontium undergo mitotic division and double in number sometime after twenty hours in the 60-day-old mice. This observation agrees with Lord (1964) who found in the young rat periodontium doubling occurred within twenty-four hours. However, his study was performed at twenty-four hour intervals so he was not able to determine a more accurate doubling time.

In the 300-day-old mice the percent labeled cells between twenty-two to forty hours is twenty-five percent greater than the period of two to twenty hours. Although the difference between these two periods is not significant, the labeled cells in the 300-day-old mice does show a tendency to double after twenty-two hours. The delay in doubling may possibly be attributable to a longer generation cycle in the periodontal ligament cells. An increase in the generation cycle has been shown in the small intestine epithelial cells of the mice, Lesher, Fry and Kohn (1961).

After a cell divides in either the young or old mouse periodontium it is no longer an old cell but there are two new "young" cells produced. The difference being less new cells are produced in the old mice. Therefore, the periodontium of aged mice has the capacity for repair

because there always is a supply of young regenerable cells. This author agrees with Tonna (1966) who studied osteogenic cells and has this same opinion. He examined fracture repair in the femur of mice and rats and found old animals with regenerable cells serving the aged adequately in periods of emergency.

CHAPTER VI

SUMMARY AND CONCLUSIONS

The periodontal ligament was studied to compare some changes occurring with age. Forty mice were divided into two groups 60 and 300-days-old. They were injected intraperitoneally with tritiated thymidine. One mouse in each group was sacrificed at two hour intervals up to forty hours after injection. Central sections were cut through the maxillary first molar and prepared as autoradiograms.

The following may be concluded from this study of the mouse periodontium:

1. The young mouse has a significantly greater number of cells.
2. The young mouse has a significantly greater number of regenerable cells.
3. The ratio of labeled to nonlabeled cells is significantly greater in the young mouse.
4. A hypothesis was proposed to explain the decreased number of cells in the aged.
5. The labeled periodontal cells undergo mitotic division

sometime after twenty hours in the 60-day-old mice.

6. A tendency for doubling to occur after twenty hours was shown in the 300-day-old mice.

7. The capacity for growth and repair is present in both the 60 and 300-day-old mice periodontium.

8. The aged periodontium has few young cells and more old cells.

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APPENDIX

TABLE I

DISTRIBUTION OF CELLS IN THE MOUSE PERIODONTIUM

	YOUNG		AGED		CONFIDENCE LEVEL
	MEAN	S.D.	MEAN	S.D.	
NUMBER OF CELLS PER SECTION	1003.9	331	689.8	140	0.001
NUMBER OF LABELED CELLS PER SECTION	3.95	3.04	1.35	1.62	0.001
PERCENTAGE OF LABELED CELLS	0.394	0.176	0.197	0.146	0.005

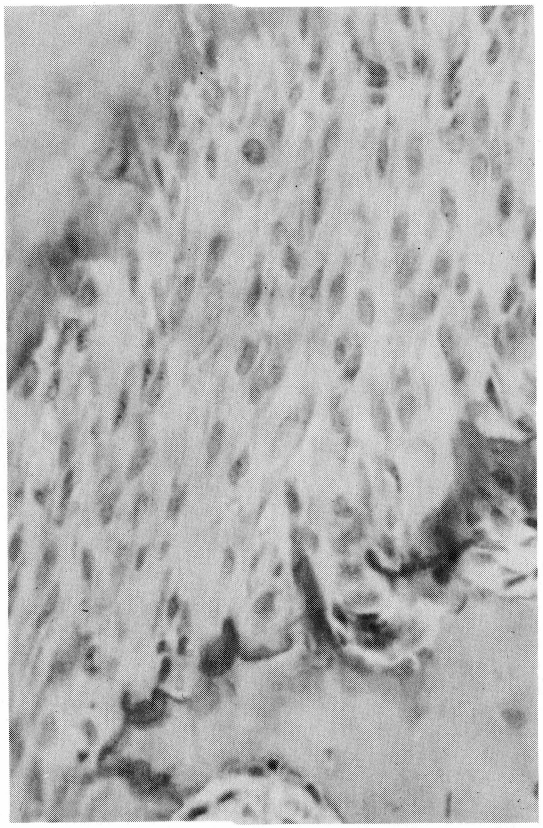

FIGURE 1

THE PERIODONTIUM OF A 60-DAY-OLD MOUSE

H&E STAIN - 400 ORIGINAL MAGNIFICATION

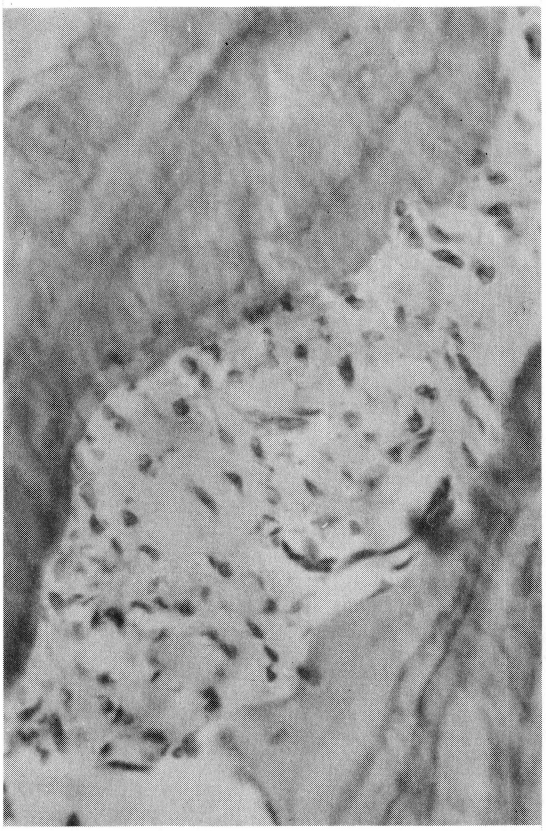

FIGURE 2

THE PERIODONTIUM OF A 300-DAY-OLD MOUSE

H&E STAIN - 400 ORIGINAL MAGNIFICATION

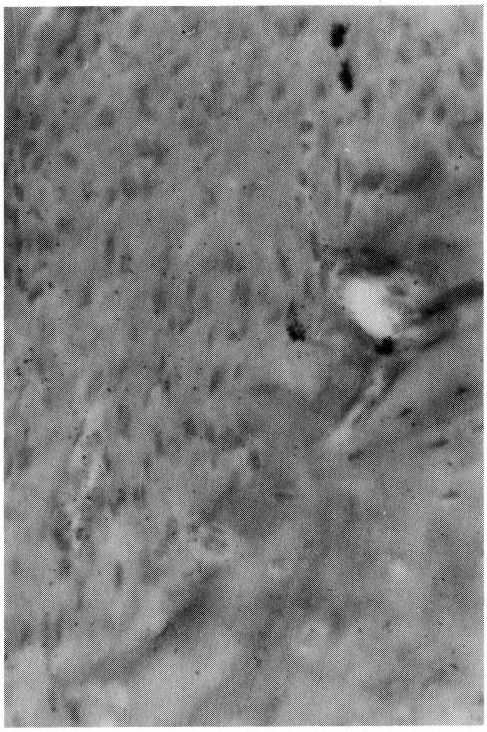

FIGURE 3

LABELED CONNECTIVE TISSUE CELLS IN THE PERIODONTIUM OF A 60-DAY-OLD MOUSE - NUCLEAR FAST RED AND INDIGO-CARMIN STAIN - 400 ORIGINAL MAGNIFICATION

FIGURE 4

LABELED CONNECTIVE TISSUE CELLS IN THE PERIODONTIUM OF A 300-DAY-OLD MOUSE - NUCLEAR FAST RED AND INDIGO-CARMIN STAIN - 400 ORIGINAL MAGNIFICATION

TABLE II

LABELING FREQUENCY IN THE MOUSE PERIODONTIUM

BEFORE AND AFTER TWENTY HOURS

	YOUNG		AGED	
	MEAN	S. D.	MEAN	S. D.
2 - 20 HOURS AFTER INJECTION PERCENT LABELED	0. 258	0. 104	0. 179	0. 143
22 - 40 HOURS AFTER INJECTION PERCENT LABELED	0. 636	0. 256	0. 241	0. 121
CONFIDENCE LEVEL	0. 005		0. 5	

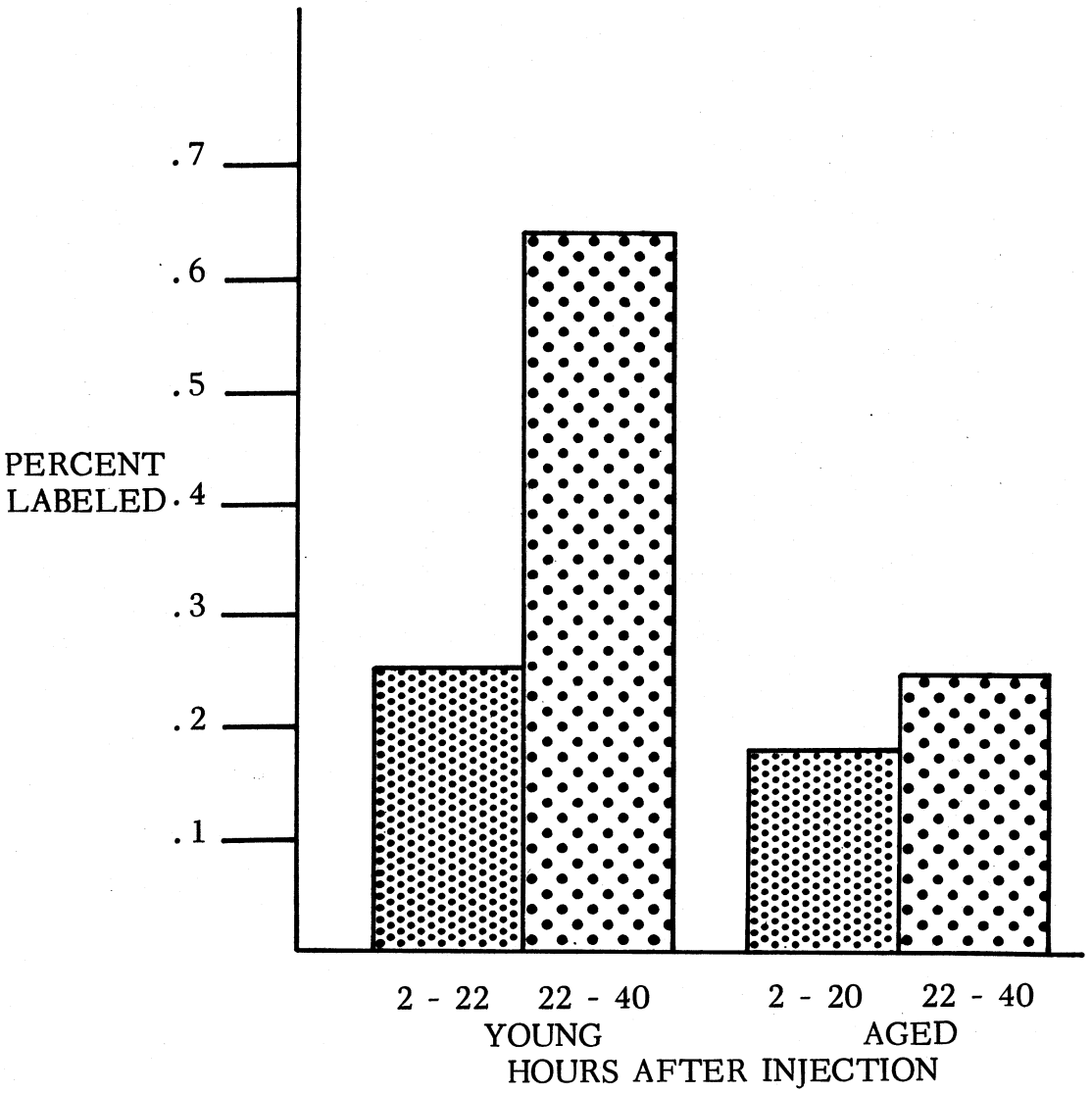

FIGURE 5

FREQUENCY DISTRIBUTION OF LABELED CELLS

APPROVAL SHEET

The thesis submitted by Dr. Melvin B. Borg has been read and approved by three members of the faculty of the Graduate School.

The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the thesis is now given final approval with reference to content, form and mechanical accuracy.

The thesis is, therefore, accepted in partial fulfillment of the requirements for the Degree of Master of Science.

DATE: May 26, 1967

Patrick D. Toto, D.D.S., M.S.

Signature of Advisor